THE BASE-PAIRING SPECIFICITY IN CHROMATOGRAPHIC SYSTEMS*

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1. Introduction

When oligonucleotides of a defined sequence are immobilised on cellulose, base pairing can take place with complementary oligonucleotides having either an homologous sequence [1–3] or an altenating one [4] according to the Watson and Crick theory. Those nucleotides undergoing such a base-pairing are adsorbed onto the cellulose and can thus be separated from any non-complementary partners present in the mixture.

It is not possible from the results reported to date, to deduce the specificity of the base-pairing in chromatographic systems. This is because it is not clear whether the base-pairing occurs between single monomer units, cooperative sequences or between the molecules as a whole. Consequently is not known whether copolymeric oligonucleotides containing complementary units in statistical distribution, will form base-pairs with their complementary partners.

In order to gain more information on this vital aspect, we have chromatographed synthetic phage DNA fragments on oligothymidine-5′-phosphate-DEAE-cellulose under the conditions for base-pairing.

2. Materials and methods

The synthesis and characterisation of the phage specific DNA fragments have been reported elsewhere [5,6] as have the preparation and application of desoxyoligo-thymidine-5′-phosphate-DEAE-cellulose PV(pT)$_n$-DEAE-cellulose [3].

* Part II of the series of 'Affinity chromatography on immobilised oligonucleotides', see ref. [3] for Part I.

The fractions within the vertical dotted lines in the elution profiles (fig. 1a–d) were combined and characterised by paper chromatography and enzymatic degradation. The results are shown in table 1.

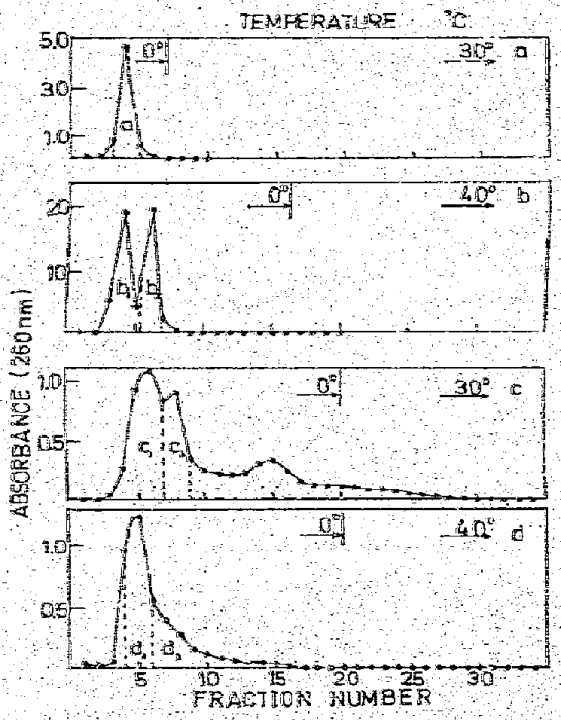

Fig. 1a–d. Affinity chromatography of phage specific DNA fragments on PV(pT)$_n$-DEAE-cellulose in NaCL buffer in the range 0° to 40°C. Column size: 23 cm × 2 cm. Fractions of 20 ml/hr are collected, those within the vertical dotted lines combined and characterised using paper chromatography (c.f. table 1). a) 120 A_{260}-units d(A-C-C-A-T-T-C-A); b) 1000 A_{260}-units d(A-G-A-A); c) 300 A_{260}-units d(A-G-A-A-A-T); d) 113 A_{260}-units d(A-G-A-A-A-T-A-A-A-A).

Table 1
Conditions for the oligonucleotide separation using column chromatography on $PV(pT)_n$-DEAE-cellulose and the characterisation of the peak fractions in figs. 1a–d

Fig. Nr.	Main product	Oligonucleotide A_{260}-units Applied	Recovered	Eluted in (Peak)	R_f-value rel. to pA	Molarity of NaCl buffer
1a	d(A-C-C-A-T-T-C-A)	120	120	120 (a)	0.34	1
1b	X*	1000	996	600 (b_1)		0.5
	d(A-G-A-A)			396 (b_2)	0.75	
1c	d(A-G-A-A-A-T)	300	240	108 (c_1)	0.41, 0.82**	0.5
	X*			20 (c_2)	0.82, 0.41**	
1d	d(A-G-A-A-A-T-A-A-A-A)	113	91	36 (d_1)	0.18	0.5
	50% main product, X*			19 (d_2)		

* Product not identified.
** Traces only.

3. Results and discussion

The synthetic phage DNA fragments d(A-C-C-A-T-T-C-A) [5] contain three adenosine units statistically distributed in the molecule. This octanucleotide is not adsorbed on the $PV(pT)_n$-DEAE-cellulose as can be seen in fig. 1a. Similarly the tetranucleotide, d(A-G-A-A) [6], which also contains three adenosine units, undergoes weak retardation but is not adsorbed (fig. 1b). This tetranucleotide can be considered in terms of Watson and Crick theory as being a complementary tetranucleotide with one failure sequence. The hexanucleotide d(A-G-A-A-A-T) [6] experiences identical retardation (fig. 1c) and can be considered as a complementary hexanucleotide containing two failure sequences. d(A-G-A-A-A-T-A-A-A) [6] is a complementary decanucleotide containing two failure sequences and is not recognized as a complementary partner by the $PV(pT)_n$-DEAE-cellulose and undergoes the same weak retardation (fig. 1d) as the other oligonucleotides. The two latter examples also show that complementary, cooperative segments which are either at one end, or in the middle of the molecule, do not give rise to any adsorption although the pure homopolymeric tri- and tetranucleotides are adsorbed [3]. In addition the elution profiles show that even after repeated purification some of the synthetic phage DNA fragments still contain impurities which are either more or less strongly retarded than the oligonucleotide fragments. The weak retardation of the synthetic oligonucleotides is probably the result of non-specific interactions with the column packing material which occur in all the cases studied here.

These results clearly show that copolymeric oligonucleotides containing the complementary monomer units in statistical distribution, undergo non-specific retardation on, but are not adsorbed as complementary partners by $PV(pT)_n$-DEAE-cellulose. Furthermore the exact position and distribution of the monomer units in the oligonucleotides is of no importance and can be purely statistical [i.e. d(*A*-C-C-*A*-T-T-C-*A*)] or be present as cooperative segments [i.e. d(A-G-*A*-*A*-*A*-T-*A*-*A*-*A*-*A*)] either at the end or in the middle of the molecule.

Thus, the base-pairing mechanism in the chromatographic system is so specific that oligonucleotides which contain only one failure sequence are no longer recognized as complementary partners and are rejected. Stable base pairs are only formed between complete complementary partners. Our findings are not in agreement with those obtained by Gilham and Robinson [7] for the separation of RNA-hydrolysates on desoxyoligothymidine-cellulose. Their results show that the number of complementary monomer units determine the degree of adsorption of statistically assembled RNA fragments. According to our results, the base-pairing in the chromatographic system also occurs according to the 'all or nothing'-principle which prohibits base-pairing between partially complementary oligonucleotides. This

principle ensures the exact reduplication of genetic information.

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